IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant : Lam, et al.
Serial No. : 09/914,543
Filed : January 17, 2002
Title : ENDOGLUCANASES

Art Unit : 1652
Examiner : Manjunath N. Rao, Ph.D.

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

DECLARATION UNDER 37 C.F.R. § 1.132

Sir:

1. I, Jay Short, am an expert in the field of molecular biology and enzyme development and was an expert at the time of the invention. I am presently employed as CEO and as a research scientist at Diversa Corporation, San Diego, CA, assignee of the above-referenced patent application. My resume is attached as documentation of my credentials.

2. I declare that the state of the art at the time of the invention and the level of skill of the person of ordinary skill in the art for screening enzymes for endoglucanase and cellulase activity was very high. Procedures for making endoglucanase and cellulase enzyme fragments and sequence variations, e.g., with substitutions, deletions, insertions, and additions, were routine in the art at the time of the invention of the above-referenced patent application. Assays for identifying endoglucanase and cellulase enzyme fragments were conventional and routine in the art at the time of the invention. Assays for identifying variant polypeptides having endoglucanase and cellulase activity were conventional and routine in the art at the time of the invention. For example, assays for identifying polypeptides having endoglucanase activity is described in the specification, e.g., on page 17, line 6 to page 18, line 7. Dye-based techniques can be used in cup-plate diffusion assays with excellent sensitivity for the determination of endoglucanase activity in culture filtrates or during enzyme purification steps (see first paragraph, page 18), as further noted in Example 1, page 36. Endoglucanase activity assays also were well known in the art at the time of the invention, e.g., as described in USPNs 4,081,328; 4,904,599; 5,110,735; 5,366,884, to list only a few examples. Many of these assays could have been adapted and used in high-throughput screening assays, which were well known in the art at

the time of the invention. Accordingly, using the teaching of the specification one of ordinary skill in the art would have been able to routinely make and use the claimed genus of nucleic acids and polypeptides without undue experimentation.

3. I declare that use of high through-put screening assays is an example of the high state of art at the time of the invention for screening polypeptides for endoglucanase and cellulase enzyme activity. For example, at the time of the invention high through-put screening assays, including *in vitro* and *in vivo* (e.g., whole cell) nucleic acid expression and enzyme screening protocols were well known in the art. As noted above, the specification sets forth exemplary endoglucanase screening assays to determine if a polypeptide is within the scope of the claimed genus – and these or many of the other well known endoglucanase and cellulase assays could have been used in high through-put screening assays. Because screening assays known in the art at the time of the invention, including high through-put enzyme screening assays, predictably gave positive results (identifying enzymes with a desired activity) it would not have taken undue experimentation to make the genus of polypeptides of the invention.

4. Additionally, I declare that methods known at the time of the invention for modifying nucleic acid and polypeptide sequences in combination with high through-put enzyme screening known at the time of the invention, made methods that require previous knowledge of protein structure, including secondary or tertiary structure, active site sequences, and the like obsolete and unnecessary. It would not have been necessary for the skilled artisan to understand which regions of the enzyme could be modified to obtain a desired activity, e.g., to generate a variant a function or activity, or, which regions of the enzyme could be modified without loss of a function or activity. It would not have been necessary for the skilled artisan to understand which specific regions of enzyme sequence or structure needed to be modified without affecting function or activity to routinely generate the claimed genus of polypeptides. Methods for making and screening sequence modifications and enzyme fragments, including high through-put enzyme screening assays, were sufficiently comprehensive, routine and predictable at the time of the invention to predictably generate endoglucanase-encoding sequences without need of knowing which specific regions of a sequence or structure affected function or activity.

Accordingly, the specification provided sufficient guidance to one of ordinary skill in the art to make the genus of polypeptides to practice the invention.

5. I declare it was considered routine to design and screen for probes of varying lengths, for example, 15, 25, 35 or 50 nucleotides in length, that could hybridize under stringent conditions to a desired polynucleotide. Additionally, the specification, e.g., on page 8, line 17, to page 9, line 5, and page 20, lines 1 to 11, give guidance to the skilled artisan how to make nucleic acids that hybridize under stringent conditions to nucleic acids of the invention.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Respectfully submitted

Date: 1/11/05

Jay Short

CURRICULUM VITAE

NAME Jay M. Short, Ph.D.

Dr. Short is a founding member of Diversa Corporation, has served as Chief Technology Officer and Director of the company since its inception in 1994. He assumed the additional roles of President in 1998 and Chief Executive Officer in 1999. In February of 2000, Dr. Short led the company's highly successful initial public offering, which raised over $200 million in gross proceeds – the largest biotechnology IPO ever completed at the time. Diversa was recently named one of the 100 most influential companies that will have the greatest influence on the future of human health. Diversa Corporation (NASDAQ: DVSA) is a leader in applying proprietary genomic technologies for the rapid discovery and optimization of novel products from genes and gene pathways.

EDUCATION

2003	Certified Director Director Training Program The Anderson Graduate School of Management, University of California, Los Angeles
1981 - 1985	Ph.D., Biochemistry Case Western Reserve University, Cleveland, Ohio
1980 - 1981	Graduate Study, Macromolecular Science Case Western Reserve University, Cleveland, Ohio
1976 - 1980	B.A. with Honors, Chemistry Taylor University, Upland, Indiana

RESEARCH & PROFESSIONAL EXPERIENCE

1999 - present	CEO and President Chief Technology Officer Board of Director Diversa Corporation San Diego, California
1998 - present	President and Chief Technology Officer Board of Director Diversa Corporation San Diego, California
1997 - 1998	Executive Vice President and Chief Technology Officer Board of Director Diversa Corporation San Diego, California
1994 - 1997	Chief Technology Officer Board of Director Diversa Corporation San Diego, California
1990 - 1994	President Stratacyte, Inc. La Jolla, California

1992 - 1994	Vice President R&D (Research) and Operations Stratagene Cloning Systems La Jolla, California
1989 - 1992	Vice President R&D (Research) and Biological Operations Stratagene Cloning Systems La Jolla, California
1988 - 1989	Senior Staff Scientist Research and Development Stratagene Cloning Systems La Jolla, California
1985 - 1988	Staff Scientist Research and Development Stratagene Cloning Systems La Jolla, California
1981 - 1985	Ph.D. Research Case Western Reserve University Dr. Richard W. Hanson's Laboratory, Identification and characterization of the promoter for P-enolpyruvate carboxykinase. First identification of a cAMP regulatory domain. Cleveland, Ohio
1980 - 1981	Graduate Student Research Case Western Reserve University Dr. Bruce Roe's Laboratory, Analysis of the cellulase activity of *Trichoderma viride.* Cleveland, Ohio

TEACHING EXPERIENCE

Thesis Advisor, University of Uppsala, Sweden, Ph.D. for Michelle Alting-Mees 1988-1993
Lecturer, Committee for Advanced Scientific Education, Center for Drug Evaluation and Research, FDA 1992
Faculty, Transgenic Mouse Model and Its Application in Assessing *In Vivo* Mutagenesis, Genetic Toxicology Workshop (3rd Annual) 1989
Microbiological Associates Inc., Bethesda, MD.
Faculty, DNA Cloning and Expression, Physiology Society Workshop, San Diego, CA. 1987
Teaching Assistant, Molecular & Cellular Biology, Case Western Reserve University, Cleveland, OH. 1981-1985
Teaching Assistant, Physiological Chemistry, Kent State University, Kent, OH. 1981
Teaching Assistant, Quantitative Analysis, Taylor University, Upland, IN. 1978-1980

CERTIFICATIONS

Certified Director	Director Training Program, University of California, Los Angeles, California The Anderson Graduate School of Management and The Harold Price Center

for Entrepreneurial Studies

PADI Diver Certification

PROFESSIONAL EXPERIENCE

Diversa ranked # 2 among small companies for one of the best places for life scientists to work in this industry.
Diversa named one of the 100 most influential companies that will have the greatest influence on the future of human health, Acumen 2004
Diversa's patent portfolio ranked # 1on the 2003 Patent Scorecard by the MIT Survey
Largest Biotechnology IPO raising over $200MM
Founding management member of Diversa Corporation

Board Director, Diversa Corporation, San Diego, CA
Board Director, Invitrogen Corporation, Carlsbad, CA
Board Director, Stressgen Biotechnologies, Vancouver, Canada and San Diego, CA
Board Director, Senomyx Corporation, San Diego, CA
Board Director, YPO (Young Presidents' Organization), San Diego, CA
Board Director & Treasurer, Stressgen Therapeutics, Victoria, BC, Canada
Board Director & Secretary, Stressgen Therapeutics, Victoria, BC, Canada
Board Director & Compensation Chairman, Victoria, BC, Canada
Board Member Advisor, Chemical and Engineering News
Board Member, BioCom San Diego
Board Advisor, IngleWood Ventures
Board of Advisors and Founding Member, Division of Biological Sciences, UCSD
Board Director and Executive Committee, Zymetrics
Fellow, Lifetime, The Explorers Club, New York, NY
Committee Member BioCom Science & Technology, San Diego
Consultant, Stratagene Cloning Systems, La Jolla, CA
Consultant, Micro Product Systems, Lynn, IN
Consultant for European Economic Community on Transgenic Toxicology Testing 1991-1994
Chairman, Discussion Group, Society of Toxicology Conference 1993
Editor, Mutation Research
Judge on the U.S. National Entrepreneur of the Year 2003
Institutional Animal Care and Use Committee (IACUC), Chairman and Institutional Official
NIEHS Peer Review Committee
Panel Member for Chemical Science & Technology for NIST, National Research Council 1997-2000
SBIR Study Section
Reviewer for U.S. Congressional Office of Technology Assessment (OTA) on The Human Genome Project and Patenting DNA Sequences.
Reviewer for Proceedings of the National Academy of Sciences, Genetic Analysis Techniques, Analytical Biochemistry & Nucleic Acids Research
U.S. Committee Member for Evaluation of Biotechnology Research in Spain
Visiting Scientist, International Centre of Insect Physiology and Ecology (ICIPE), Kenya 2002-2004

MEMBERSHIPS

American Association for the Advancement of Science
American Chemical Society
American Men and Women of Science
American Society of Biochemistry and Molecular Biology

American Society of Microbiology
BioCom San Diego
Environmental Mutagenesis Society
Japanese Environmental Mutagen Society
Science
Society for Industrial Microbiology
Society of Toxicology
The Explorers Club, Fellow Lifetime Member, New York
The New York Academy of Sciences
YPO (Young Presidents' Organization) San Diego
YPO (Young Presidents' Organization) International

AWARDS

Henry F. Whalen, Jr. Award for Business Development, American Chemical Society, 2004
Distinguished Alumnus Award for Professional Achievement, Taylor University, Upland, IN 2004
Taylor University nomination for CCCU Award (Council for Christian Colleges & Universities) 2003
Case Western Reserve University Alumni Profile 2003
bioFusion 03 Breakthrough Innovation in Science Award Nomination 2003
bioFusion 03 Life Science Leader of the Year Nomination 2003
bioFusion 03 Life Science Company of the Year Nomination 2003
ABL (Adaptive Business Leader) Innovations in HealthCare Gold Award 2003
Deloitte and Touche's Orange County / San Diego Technology "Fast 50" 2003
Finalists for UCSD Connect's Most Innovative New Product Award in the Biotechnology R&D Category 2002
Deloitte and Touche "Fast 500" Technology 2002
Deloitte and Touche's Orange County / San Diego Technology "Fast 50" 2002
The Premier Print Award, Annual Report 2002
Deloitte and Touche "Fast 500" Technology 2001
Ernst & Young San Diego Entrepreneur of the Year 2001
bioFusion 01 Life Science Innovator Award Nomination 2001
T-Sector Life Science Innovator Award 2001
Deloitte and Touche's Orange County / San Diego Technology "Fast 50" 2001
San Diego Business Journal StarCom Honor 2001
League of American Communication Professionals, Platinum Award, Annual Report 2001
Ernst & Young Finalist for San Diego Entrepreneur of the Year in 2000
The Premier Print Award, Annual Report 2001
American Men and Women of Science 1995
Who's Who Registry of Business Leaders 1994-1995
SBIR Annual Report Program Success Profile (Top 8 of 800 Companies) 1993
Stratagene Most Innovative Award - Managers/Supervisors 1992
Stratagene Innovation Award - Big Blue® Transgenic Testing System 1991
UCSD Connect Program 1st Place Award for Innovation and Entrepreneurship in Biotechnology 1991
UCSD Connect Program 1st Place Award for Innovation and Entrepreneurship in Biotechnology 1990
Stratagene Innovation Award - Lambda ZAP® vector 1990
Stratagene Service Award 1990
Award from the University of Victoria for Contributions to the Development of Short-term Transgenic Mutation Assays
Nominated as Council Member for the U.S. Environmental Mutagen Society
PNIT Patent Award

MEDIA:

ABC Discovery News, ABC San Diego Channel 10, Agricultural Genomics, BBC Radio, Billings Gazette, BioCentury, Bioinformed Newsletter, BioPeople Magazine, BioTech Today Radio Show, Biotechnology Newsletter, BioVentures View, BioWorld Today, Business Daily, Business Week, CBS MarketWatch Weekend, CEO Cast, Chemical Engineering, Chemical Week, Chemistry & Industry (UK), Chemistry, CNBC, CNN Science & Technology, CNN Sunday Weekend, CNN WorldView, dBusiness.com, Digital Jam, Discovery Magazine, Drug Discovery Today, Elsevier Science Ltd., Forbes, Forbes.com, Fox CONNECT, Fox 6 News San Diego, German RTL TV, Good Morning America, Horizon Air Magazine, Idea TV, Inside Business Radio Show, JAG Financial News, KCRA Channel 3, KBPS Radio, KFMB Channel 8, KGTV Channel 10, KPBS, KUSI, Life Technology, London Financial Times, Los Angeles Times, Modern Drug Discovery, NBC San Diego Channel 7/39, National Geographic, National Radio Report, Nature, Nature Biotechnology, New York Times, PBS, Pirateinvestor.com, R&D Magazine, Reuters, San Diego Business Journal, San Diego Business Transcript, San Diego Magazine, San Diego Metropolitan, San Diego Union Tribune, SIM, Scientist, Specialty Chemicals, Sp2 Magazine, Stewards' Watch, T-Sector Magazine, The Age Magazine, The Economist, The Motley Fool, The Discovery Channel, The Discovery Channel, Time Magazine, USA Today, Wall Street Journal, Wall Street Transcript, Washington Post

PATENTS

The Patent Scorecard for 2003 recognized Diversa's patent portfolio as being ranked # 1 by the MIT Survey. This ranking provides an overall assessment of a company's intellectual property power. This measure showcases the broader significance of a company's patents by examining how often its U.S. patents from the previous five years are cited as prior art in the current year's batch. A value of 1.0 represents average citation frequency, so, for example, a value of 1.4 would indicate a company's patents were cited 40 percent more often than the average. Diversa has a value of 14.43.

DNA Cloning Vectors with *in vivo* Excisable Plasmids 1987
Mutagenesis Testing Using Transgenic Animals Carrying Marker Genes 1987
Mutagenesis Testing Using Transgenic Non-Human Animals Carrying Test DNA Sequences 1987
Dietary and Hormonal Regulation of Expression of Exogenous Genes in Transgenic Animals Under Control of the Promoter of the Gene
Phosphoenolpyruvate Carboxykinase 1988
A Transgenic Mouse for Measurement and Characterization of Mutation Induction *In Vivo* 1989
Rapid Screening Mutagenesis and Teratogenesis Assay 1989
A Combinatorial Approach to Regenerating the Immunoglobulin Repertoire in Prokaryotic Cells 1990
Transgenic Animal Models for *In Vivo* Mutagenesis Testing 1990
Polycos Vectors 1991
A Lambda Packaging Extract Lacking β-Galactosidase Activity 1991
A System for Regulation of Eukaryotic Genes 1991
Methods for Phenotype Creation from Multiple Gene Populations 1991
Transgenic Non-Human Animals Carrying Test DNA Sequences 1992
Mutagenesis Testing Using Transgenic Non-Human Animals Carrying Test DNA Sequences 1992
Selectable System Patent 1992
Polycos Mutagenesis Systems 1993
Use of Trans-acting Proteins for the Development of an *In Situ* Expression Screening System 1993
Enzyme Kits and Libraries 1995
Enzyme Activity Screening of Clones having DNA from Uncultivated Microorganisms 1995
Enzyme Tiered 1995
Method for Screening for Enzyme Activity 1995
Combined Enzyme Screening/Evolution 1995
Uncultured/Activity Screening 1995
Directed Evolution of Thermophilic Proteins 1995
Combinatorial Enzyme Development (Directed Mutagenesis) 1996
Protein Activity Screening of Clones having DNA from Uncultivated Microorganisms 1996
Production and Use of Normalized DNA Libraries 1996

Methods of DNA Shuffling with Polynucleotides Produced by Blocking or Interrupting a Synthesis or Amplification Process 1996
Method of Screening for Enzyme Activity (Biopanning) 1996
Directed Evolution of Thermophilic Enzymes 1996
Environmental Biopanning 1996
Combinatorial Enzyme Development 1996
Enzyme Activity Screening of Clones Having DNA from Uncultivated Microorganisms 1996
Normalized Samples/Libraries 1996
Reassembled Pools of Mutagenized DNA & Procedure 1996
Fluorescent-based Single Screening for Enzymes 1996
High Throughput Screening for Novel Enzymes 1997
Nucleotide Sequence of the *Aquifex aeolicus* Genome, Fragments Thereof, and Uses Thereof 1997
Screening for Novel Bioactivities 1997
Screening for Novel Compounds which Regulate Biological Interactions 1997
Method for Screening Enzyme Activity 1997
High Throughput Screening for Novel Enzymes 1997
"Discovery" (whole process, including uncultivated, normalized, biopanning, screening, evolving, (etc.) 1997
Production of Enzymes Having Desired Activities By Mutagenesis 1999
Protein Activity Screening of Clones Having DNA from Uncultivated Microorganisms 1999
Method of DNA Reassembly by Interrupting Synthesis 1999
Production and Use of Normalized DNA Libraries 1999
Enzyme Kits and Libraries 1999
Screening for Novel Bioactivities 2000
Method for Screening for Enzyme Activity 2000
Screening for Novel Bioactivities 2000
Production and Use of Normalized DNA Libraries 2000
Method of Screening for Enzyme Activity 2000
Screening Methods for Enzymes and Enzyme Kits 2001
Saturation Mutagenesis in Directed Evolution 2001
High Throughput Screening for Novel Enzymes 2001
Recombinant Bacterial Phytases and Uses Thereof 2001
Methods Useful for Nucleic Acid Sequencing Using Modified Nucleotides Comprising Phenylboronic Acid 2001
End Selection in Directed Evolution 2001
Gene Expression Library Produced From DNA From Uncultivated Microorganisms and Method for Making the Same 2001
Directed Evolution of Thermophilic Enzymes 2002
Method for Screening for Enzyme Activity 2002
Exonuclease-Mediated Gene Assembly in Directed Evolution 2002
End Selection In Directed Evolution 2002
Exonuclease-Mediated Gene Assembly in Directed Evolution 2002
Screening for Novel Bioactivities 2002
Method of DNA Shuffling with Polynucleotides Produced or Blocking or Interrupting Synthesis or Amplification Process 2002
Production and Use of Normalized DNA Libraries 2002
Sequence Based Screening 2002
Non-Stochastic Generation of Genetic Vaccines 2002
Altered Thermostability of Enzymes 2003
Screening Methods for Enzymes and Enzyme Kits 2003
Methods for Identifying a Desired Enzymatic Activity 2003
Enzymes Kits and Libraries 2003
Method for Screening for Enzyme Activity 2003
Protein Activity Screening of Clones having DNA from Uncultivated Microorganisms 2003
High Throughput Screening of Mycelia for Bioactivities of Biomolecules 2003
Screening for Novel Bioactivities 2003
Coated Surfaces for Selective Enrichment of Microbial Populations 2003

Recombinant Bacterial Phytases and Uses Thereof 2003
Synthetic Ligation Reassembly in Directed Evolution 2003
Process for Generating Optimized Molecules from a Manmade Library of Polynucleotides made by Combinatorial Saturation Mutagenesis (amended) 2003
Exonuclease-Mediated Nucleic Acid and Reassembly in Directed Evolution 2003
Methods for Purifying Annealed Doubled-Stranded Oligonucleotides Lacking Base Pair Mismatches 2004
End Selection in Directed Evolution 2004
Protein Activity Screening of Clones having DNA from Uncultivated Microorganisms 2004
Method of Screening for Enzyme Activity 2004
Exonuclease-Mediated Gene Assembly in Directed Evolution (3/23/04 new issuance) 2004
Directed Evolution of Thermophilic Enzymes (3/30/04 new issuance) 2004
Non-Stochastic Generation of Genetic Vaccines and Enzymes 2004
Directed Evolution of Thermophilic Enzymes 2004
Over 350 Additional Pending Patent Applications Worldwide.

GRANTS AND CONTRACTS

*Phase I Small Business Contract #N43-Am-62282. 1985-1986 P.I.
Vectors and Techniques for Rapid DNA Sequencing
*Phase II Small Business Contract #N43-Am-62282. 1988-1990 P.I.
Vectors and Techniques for Rapid DNA Sequencing
*Phase I Small Business Grant 2R43ES04484-02. 1986-1987 P.I.
Identification of Genetic Lesions Leading to Mutations
*Phase II Small Business Grant 2R43ES04484-02. 1989-1992 P.I.
Identification of Genetic Lesions Leading to Mutations
*1R01-ES04728-01A1. 1989-1992. (NIEHS) P.I.
Animal Model for Identification of Genetic Lesions
*Phase I Small Business Grant #R43GM42291-01. 1989 P.I.
Switch Mechanism for Gene Expression in Transgenics
*RFP NIH-ES-88-11. 1989-1994. (NIEHS) Co-I.
Development of Mutagenesis Assays Using Transgenic Mice
*Phase II Small Business Grant #2R44GM42291-02. 1990-1992 (DRG/NIH) P.I.
Switch Mechanism for Gene Expression in Transgenics
*Phase I Small Business Grant #1R43GM46585-01. 1991 (DRG/NIH) P.I.
Generation of a Peptide Screening System Through the Development of Combinatorial-splicing "Polycos" Vectors
*Phase I Small Business Grant #1R43CA57066-01. 1992 (NCI) P.I.
Transgenic Rats: A Short-term Mutagenicity Assay for Multi-species Testing of Suspected Human Carcinogens
*Phase I Small Business Grant #1R43GM48300-01. 1992. (DRG/NIH) P.I.
Analysis of the Immunoglobulin Hypermutator Mechanism
*Phase I Small Business Grant #1R43ES06146-01. 1992 (NIEHS) P.I.
Selectable "Polycos" Shuttle Vectors for In Vivo Mutagenicity Testing
*Phase II Small Business Grant #2R44GM46585-02. 1992-1994 (NIGMS) P.I.
Peptide Screening Utilizing Combinatorial Polycos Vector
*Phase I Small Business Grant #1R43RR08667-01. 1992-1993 (DRG/NIH) Co-I.
A One-step PCR Cloning System Based on FLP Recombination
*Phase II Small Business Grant #2R44CA57066-02. 1993-1995 (NCI) P.I.
Transgenic Rats:Transgenic Rat Model for Mutagenicity Testing
*Phase I Small Business Grant. 1993-1994 (NIH) Co-I.
Transgenic Fish Model for Mutagenicity Testing
*Phase II Small Business Grant 1994-1996 (NIH) P.I.

"Polycos" Shuttle Vectors for Mutagenicity testing
*Phase I Small Business Grant. 1994 (NIH) Co-I.
Vector System for Studying Protein-Protein Interactions
*CRADA with LLNL. 1994 (NIH) Co-I.
Mouse and Rat Painting Probes
*CRADA with FDA. 1994 (NIH) Co-I.
Tamoxifen Testing in F-344 Rats
*CRADA with NASA. 1994 (NIH) Co-I.
Radiation Damage in the Microgravity Environment

ABSTRACTS AND INVITED LECTURES:

Over 200 Abstracts and Invited Lectures.

PUBLICATIONS:

1. Yoo-Warren, H., Monahan, J.E., Short, J.M., Short, H., Bruzel, A., Wynshaw-Boris, A., Meisner, H.M., Samols, D., and Hanson, R.W. (1983) Isolation and Characterization of the Gene Coding for Cytosolic Phosphoenolpyruvate Carboxykinase (GTP) from the Rat. *Proc. Natl. Acad. Sci. U.S.A.,* 80:3656-3660.

2. Wynshaw-Boris, A., Lugo, T.G., Short, J.M., Fournier, R.E.K., and Hanson, R.W. (1984) Identification of cAMP Regulatory Region in the Gene for Rat Cytosolic Phosphoenolpyruvate Carboxykinase (GTP): Use of Chimeric Genes Transfected into Hepatoma Cells. *J. Biol. Chem.,* 259:12161-12169.

3. Wynshaw-Boris, A., Lugo, T.G., Short, J.M., Fournier, R.E.K., and Hanson, R.W. (1985) A Region of the Gene for Rat Cytosolic P-enolpyruvate Carboxykinase Confers cAMP Responsiveness to the HSV-thymidine Kinase Gene. In: *Membrane Receptors and Cellular Recognition,* (M. Czech and C.R., Kahn, eds.), Alan Liss Inc., New York, pp 339-346.

4. Wynshaw-Boris, A., Short, J.M., and Hanson, R.W. (1986) Characterization of the Phosphoenolpyruvate Carboxykinase (GTP) Promoter-Regulatory Region. I. Multiple Hormone Regulatory Elements and the Effects of Enhancers. *J. Biol. Chem.,* 261:9714-9720.

5. Short, J.M., Wynshaw-Boris, A., Short, H.P., and Hanson, R. W. (1986) Characterization of the Phosphoenolpyruvate Carboxykinase (GTP) Promoter-Regulatory Region. II. Identification of cAMP and Glucocorticoid Regulatory Domains. *J. Biol. Chem.,* 261:9721-9726.

6. Wynshaw-Boris, A., Short, J.M., and Hanson, R.W. (1986) The Determination of Sequence Requirements for Hormonal Regulation of Gene Expression. *Biotechniques*, 4:104-119.

7. Burns, D.M., Bhandari, G., Short, J.M., Sanders, P.G., Wilson, R.H., and Miller, R.E. (1986) Selection of a Rat Glutamine Synthetase cDNA Clone. *Biochemical and Biophysical Research Communications,* 134:146-151.

8. Hod., Y. Cook, J.S., Weldon, S.L., Short, J.M., Wynshaw-Boris, A., and Hanson, R.W. (1986) Differential Expression of the Genes for the Mitochondrial and Cytosolic Forms of P-enolpyruvate Carboxykinase Gene. In: *Metabolic Regulation: Application of Recombinant DNA Techniques,* (A.G., Goodridge and R.W. Hanson eds.), Annals of the New York Academy of Sciences, New York, Vol. 278, pp. 31-45.

9. Wynshaw-Boris, A., Short, J.M., and Hanson, R.W. (1987) *cis* - acting Regulatory Elements in Hormonally Responsive Genes. In: *Progress in Nucleic Acid Research and Molecular Biology* (W.E. Cohn and K. Moldave eds.), Academic Press, Inc., Orlando, Florida, 34:59-87.

10. Bullock, W., Fernandez, J.M., and Short, J.M. (1987) XL1-Blue: A High Efficiency Plasmid Transforming *rec*A *E.coli* Strain With β-Galactosidase Selection. *Biotechniques*, 5:60-64.

11. Short, J.M., Fernandez, J.F., Sorge, J.A., and Huse, W. (1988) Lambda ZAP®: A Bacteriophage Lambda Expression Vector With *In Vivo* Excision Properties. *Nucleic Acids Res.,* 16:7583-7600.

12. Short, J.M. (1988) Book Review: Vectors - A Survey of Molecular Cloning Vectors and Their Uses. Raymond L. Rodriques and David T. Denhardt, eds, Butterworths, Stoneham, MA. *Genomics*, 2:270-271.

13. Short, J.M., and Pollard, A. (1988) Gigapack XL: Size Selective Packaging Extract. *Strategies in Mol. Biol.*, 1:5-7.

14. Kretz, P.L., and Short, J.M. (1989) Gigapack II: A Restriction Deficient (*mcrA-, B-, hsd-, mrr-*) Lambda Packaging Extract. *Strategies in Mol. Biol.*, 2(2):25-26.

15. Kretz, P.L., Reid, C.H., Greener, A., and Short, J.M. (1989) Effect of Lambda Packaging Extract Mcr Restriction Activity on DNA Cloning. *Nucleic Acids Res.* 17:5409.

16. Sastry, L., Alting-Mees, M., Huse, W.D., Short, J.M., Sorge, J.A., Hay, B.N., Janda, K.D., Benkovic, S.J., and Lerner, R.A. (1989) Cloning of the Immunological Repetoire in *E. coli* for Generation of Monoclonal Catalytic Antibodies I. Construction of a V_H Specific cDNA Library. *Proc. Natl. Acad. Sci. U.S.A.,* 86:5728-5732.

17. Short, J.M. (1989) The Use of Lambda Phage Shuttle Vectors in Transgenic Mice for Development of a Short Term Mutagenicity Assay. In: *Fifth International Conference on Environmental Mutagens*, Alan Liss, Inc., New York,Part A:335-367. Article and Lecture.

18. Alting-Mees, M., and Short, J.M. (1989) pBluescript II: Gene Mapping Vectors. *Nucleic Acids Res.,* 17:9494.

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20. Alting-Mees, M., Amberg, J., Ardourel, D., Elgin, E., Greener, A., Gross, E.A., Kubitz, M., Mullinax, R.L., Short, J.M., and Sorge, J.A. (1990) Monoclonal Antibody Expression Libraries:A Rapid Alternative to Hybridomas. *Strategies in Mol. Biol.*, 3:1-9.

21. Kohler, S., Provost, S., Dycaico, M., Sorge, J., and Short, J.M. (1990) Development of a Short-term, *In Vivo* Mutagenesis Assay: The Effects of Methylation on the Recovery of a Lambda Phage Shuttle Vector from Transgenic Mice. *Nucleic Acids Res.,* 18:3007-3013.

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JOURNAL Appl. Microbiol. Biotechnol. 46 (5-6), 538-544 (1996)
MEDLINE 97161783
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AUTHORS Kitamoto,N.
TITLE Direct Submission
JOURNAL Submitted (29-FEB-1996) Noriyuki Kitamoto, Food Reseach Institute, Aichi Prefectural Government; Shinpukiji-cho 2-1-1, Nishi-ku, Nagoya, Aichi 451, Japan (Tel:052-521-9316, Fax:052-532-5791)
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  TITLE     Direct Submission
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Brief Summary GI List ASN.1 GenPept GenPept(Full) FASTA XML GPSeq XML TinySeq XML Graph all to file this page to text selected items to clipboard
Range: from to Features: SNP CDD MGC HPRD
1: CAA93273. Reports endo-1,4-beta-glu...[gi:1297061] BLink, Domains, Links

LOCUS CAA93273 257 aa linear BCT 29-JAN-1997
DEFINITION endo-1,4-beta-glucanase [Thermotoga maritima].
ACCESSION CAA93273
VERSION CAA93273.1 GI:1297061
DBSOURCE embl locus TMCELAB, accession Z69341.1
KEYWORDS .
SOURCE Thermotoga maritima
ORGANISM Thermotoga maritima
Bacteria; Thermotogae; Thermotogales; Thermotogaceae; Thermotoga.
REFERENCE 1 (residues 1 to 257)
AUTHORS Liebl,W., Ruile,P., Bronnenmeier,K., Riedel,K., Lottspeich,F. and Greif,I.
TITLE Analysis of a Thermotoga maritima DNA fragment encoding two similar thermostable cellulases, CelA and CelB, and characterization of the recombinant enzymes
JOURNAL Microbiology 142, 2532-2542 (1996)
REFERENCE 2 (residues 1 to 257)
AUTHORS Liebl,W.
TITLE Direct Submission
JOURNAL Submitted (02-FEB-1996) Liebl W., Technische Universitaet Muenchen, Lehrstuhl f. Mikrobiologie, Arcisstr. 21, 80290 Muenchen, Federal Republic of Germany
FEATURES Location/Qualifiers
source 1..257
/organism="Thermotoga maritima"
/strain="MSB8 (DSM3109)"
/db_xref="taxon:2336"
Protein 1..257
/product="endo-1,4-beta-glucanase"
/EC_number="3.2.1.4"
/function="hydrolysis of carboxymethyl cellulose, beta-glucan, etc."
CDS 1..257
/gene="celA"
/standard_name="cellulase"
/coded_by="Z69341.1:1107..1880"
/citation=[1]
/evidence=experimental
/transl_table=11
/db_xref="GOA:Q60032"
/db_xref="UniProt/TrEMBL:Q60032"
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61 yvlgypefyy gykpwenhta egsklpvpvs smksfsvevs fdihhepslp lnfametwlt
121 rekyqteasi gdveimvwfy fnnltpggek ieeftipfvl ngesvegtwe lwlaewgwdy

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      241 qfgwkfenfs idlevre
//
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LOCUS P22669 237 aa linear PLN 25-JAN-2005
DEFINITION Endoglucanase I precursor (Endo-1,4-beta-glucanase) (Cellulase) (FI-CMCase).
ACCESSION P22669
VERSION P22669 GI:121835
DBSOURCE swissprot: locus GUN_ASPAC, accession P22669;
class: standard.
created: Aug 1, 1991.
sequence updated: Aug 1, 1991.
annotation updated: Jan 25, 2005.
xrefs: gi: 217818, gi: 217819, gi: 2287, gi: 2288, gi: 101743
xrefs (non-sequence databases): HSSPO74705, InterProIPR008985, InterProIPR002594, PfamPF01670, ProDomPD004316
KEYWORDS Cellulose degradation; Direct protein sequencing; Glycosidase; Hydrolase; Pyrrolidone carboxylic acid; Signal.
SOURCE Aspergillus aculeatus
ORGANISM Aspergillus aculeatus
Eukaryota; Fungi; Ascomycota; Pezizomycotina; Eurotiomycetes; Eurotiales; Trichocomaceae; mitosporic Trichocomaceae; Aspergillus.
REFERENCE 1 (residues 1 to 237)
AUTHORS Ooi,T., Shinmyo,A., Okada,H., Murao,S., Kawaguchi,T. and Arai,M.
TITLE Complete nucleotide sequence of a gene coding for Aspergillus aculeatus cellulase (FI-CMCase)
JOURNAL Nucleic Acids Res. 18 (19), 5884 (1990)
PUBMED 2216782
REMARK NUCLEOTIDE SEQUENCE.
STRAIN=F-50
REFERENCE 2 (residues 1 to 237)
AUTHORS Ooi,T., Shinmyo,A., Okada,H., Hara,S., Ikenaka,T., Murao,S. and Arai,M.
TITLE Cloning and sequence analysis of a cDNA for cellulase (FI-CMCase) from Aspergillus aculeatus
JOURNAL Curr. Genet. 18 (3), 217-222 (1990)
PUBMED 2249253
REMARK NUCLEOTIDE SEQUENCE, AND PARTIAL PROTEIN SEQUENCE.
STRAIN=F-50
COMMENT [CATALYTIC ACTIVITY] Endohydrolysis of 1,4-beta-D-glucosidic linkages in cellulose, lichenin and cereal beta-D-glucans.
[SUBCELLULAR LOCATION] Secreted.
[INDUCTION] By cellulosic materials and hemicelluloses.
[MISCELLANEOUS] Will also hydrolyze 1,4-linkages in beta-D-glucans also containing 1,3-linkages.
[SIMILARITY] Belongs to the glycosyl hydrolase 12 (cellulase H) family.

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FEATURES             Location/Qualifiers
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                     /db_xref="taxon:5053"
     Protein         1..237
                     /product="Endoglucanase I precursor"
                     /EC_number="3.2.1.4"
     Region          1..16
                     /region_name="Signal"
                     /note="Potential."
                     /evidence=experimental
     Region          17..237
                     /region_name="Mature chain"
                     /note="Endoglucanase I."
                     /evidence=experimental
     Site            17
                     /site_type="modified"
                     /note="Pyrrolidone carboxylic acid."
                     /evidence=experimental
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       61 tswstkwnws ggensvksya nsgltfnkkl vsqisqiptt arwsydntgi radvaydlft
      121 aadinhvtws gdyelmiwla ryggvqpigs qiatatvdgq twelwygang sqktysfvap
      181 tpitsfqgdv ndffkyltqn hgfpassqyl itlqfgtepf tggpatlsvs nwsasvq
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LOCUS P16630 264 aa linear BCT 25-OCT-2004
DEFINITION Endoglucanase S precursor (Endo-1,4-beta-glucanase S) (Cellulase S).
ACCESSION P16630
VERSION P16630 GI:121830
DBSOURCE swissprot: locus GUNS_ERWCA, accession P16630;
class: standard.
created: Aug 1, 1990.
sequence updated: Aug 1, 1990.
annotation updated: Oct 25, 2004.
xrefs: gi: 148389, gi: 148390, gi: 95575
xrefs (non-sequence databases): InterProIPR008985, InterProIPR002594, PfamPF01670, ProDomPD004316
KEYWORDS Cellulose degradation; Direct protein sequencing; Glycosidase; Hydrolase; Signal.
SOURCE Pectobacterium carotovorum
ORGANISM Pectobacterium carotovorum
Bacteria; Proteobacteria; Gammaproteobacteria; Enterobacteriales; Enterobacteriaceae; Pectobacterium.
REFERENCE 1 (residues 1 to 264)
AUTHORS Saarilahti,H.T., Henrissat,B. and Palva,E.T.
TITLE CelS: a novel endoglucanase identified from Erwinia carotovora subsp. carotovora
JOURNAL Gene 90 (1), 9-14 (1990)
MEDLINE 90337352
PUBMED 2379837
REMARK SEQUENCE FROM N.A., AND PARTIAL SEQUENCE.
STRAIN=SCC3193
COMMENT ----------
This SWISS-PROT entry is copyright. It is produced through a collaboration between the Swiss Institute of Bioinformatics and the EMBL outstation - the European Bioinformatics Institute. The original entry is available from http://www.expasy.ch/sprot and http://www.ebi.ac.uk/sprot
----------.
[CATALYTIC ACTIVITY] Endohydrolysis of 1,4-beta-D-glucosidic linkages in cellulose, lichenin and cereal beta-D-glucans.
[SIMILARITY] Belongs to the glycosyl hydrolase 12 (cellulase H) family.

FEATURES Location/Qualifiers
source 1..264
/organism="Pectobacterium carotovorum"
/db_xref="taxon:554"
gene 1..264
/gene="celS"

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                     /product="Endoglucanase S precursor"
                     /EC_number="3.2.1.4"
     Region          1..32
                     /gene="celS"
                     /region_name="Signal"
                     /evidence=experimental
     Region          33..264
                     /gene="celS"
                     /region_name="Mature chain"
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      121 itsnvtysik atgtynaayd iwfhttdkan wdssptdelm iwlndtnagp agdyietvfl
      181 gdsswnvfkg winadngggw nvfsfvhtsg tnsaslnirh ftdylvqtkq wmsdekyiss
      241 vefgteifgg dgqiditewr vdvk
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LOCUS 2NLR_A 234 aa linear BCT 02-NOV-1998
DEFINITION Chain A, Streptomyces Lividans Endoglucanase (Ec: 3.2.1.4) Complex With Modified Glucose Trimer.
ACCESSION 2NLR_A
VERSION 2NLR_A GI:6573594
DBSOURCE pdb: molecule 2NLR, chain 65, release Nov 2, 1998;
deposition: Nov 2, 1998;
class: Hydrolase;
source: Mol_id: 1; Organism_scientific: Streptomyces Lividans;
Strain: 66; Gene: CelB; Expression_system: Streptomyces Lividans;
Expression_system_strain: 66; Expression_system_plasmid: Piaf9, Piaf18;
Exp. method: X-Ray Diffraction.
KEYWORDS .
SOURCE Streptomyces lividans
ORGANISM Streptomyces lividans
Bacteria; Actinobacteria; Actinobacteridae; Actinomycetales; Streptomycineae; Streptomycetaceae; Streptomyces.
REFERENCE 1 (residues 1 to 234)
AUTHORS Wittmann,S., Shareck,F., Kluepfel,D. and Morosoli,R.
TITLE Purification and characterization of the CelB endoglucanase from Streptomyces lividans 66 and DNA sequence of the encoding gene
JOURNAL Appl. Environ. Microbiol. 60 (5), 1701-1703 (1994)
MEDLINE 94288649
PUBMED 8017952
REFERENCE 2 (residues 1 to 234)
AUTHORS Sulzenbacher,G., Shareck,F., Morosoli,R., Dupont,C. and Davies,G.J.
TITLE The Streptomyces lividans family 12 endoglucanase: construction of the catalytic cre, expression, and X-ray structure at 1.75 A resolution
JOURNAL Biochemistry 36 (51), 16032-16039 (1997)
MEDLINE 98101362
PUBMED 9440876
REFERENCE 3 (residues 1 to 234)
AUTHORS Sulzenbacher,G., Mackenzie,L.F., Wilson,K.S., Withers,S.G., Dupont,C. and Davies,G.J.
TITLE The crystal structure of a 2-fluorocellotriosyl complex of the Streptomyces lividans endoglucanase CelB2 at 1.2 A resolution
JOURNAL Biochemistry 38 (15), 4826-4833 (1999)
MEDLINE 99218092
PUBMED 10200171
REFERENCE 4 (residues 1 to 234)
AUTHORS Sulzenbacher,G., Dupont,C. and Davies,G.J.
TITLE Direct Submission
JOURNAL Submitted (02-NOV-1998)

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COMMENT     Revision History:
            FEB 7 0 Typographical
            DEC 8 99 Typographical
            NOV 12 99 Typographical
            NOV 10 99 Initial Entry.
FEATURES             Location/Qualifiers
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                     /db_xref="taxon:1916"
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                     /bond_type="disulfide"
     SecStr          9..14
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                     /note="strand 2"
     SecStr          16..21
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                     /note="strand 3"
     SecStr          22..25
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                     /note="strand 4"
     SecStr          28..36
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                     /note="strand 5"
     SecStr          37..47
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                     /note="strand 6"
     SecStr          54..59
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     SecStr          60..63
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                     /note="strand 8"
     Bond            bond(64,69)
                     /bond_type="disulfide"
     SecStr          67..70
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                     /note="strand 9"
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     SecStr          211..215
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                     /note="strand 22"
     SecStr          216..221
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                     /note="strand 23"
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       61 fngchytncs pgtdlpvrld tvsaapssis ygfvdgavyn asydiwldpt artdgvnqte
      121 imiwfnrvgp iqpigspvgt asvggrtwev wsggngsndv lsfvapsais gwsfdvmdfv
      181 ratvarglae ndwyltsvqa gfepwqngag lavnsfsstv etgtpggtdp gdpg
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Example 14: Product by Function

Specification: The specification exemplifies a protein isolated from liver that catalyzes the reaction of A → B. The isolated protein was sequenced and was determined to have the sequence as set forth in SEQ ID NO: 3. The specification also contemplates but does not exemplify variants of the protein wherein the variant can have any or all of the following: substitutions, deletions, insertions and additions. The specification indicates that procedures for making proteins with substitutions, deletions, insertions and additions is routine in the art and provides an assay for detecting the catalytic activity of the protein.

Claim:

A protein having SEQ ID NO: 3 and variants thereof that are at least 95% identical to SEQ ID NO: 3 and catalyze the reaction of A → B.

Analysis:

A review of the full content of the specification indicates that a protein having SEQ ID NO: 3 or variants having 95% identity to SEQ ID NO: 3 and having catalytic activity are essential to the operation of the claimed invention. The procedures for making variants of SEQ ID NO: 3 are conventional in the art and an assay is described which will identify other proteins having the claimed catalytic activity. Moreover, procedures for making variants of SEQ ID NO: 3 which have 95% identity to SEQ ID NO: 3 and retain its activity are conventional in the art.

A review of the claim indicates that variants of SEQ ID NO: 3 include but are not limited to those variants of SEQ ID NO: 3 with substitutions, deletions, insertions and additions; but all variants must possess the specified catalytic activity and must have at least 95% identity to the SEQ ID NO: 3. Additionally, the claim is drawn to a protein which **comprises** SEQ ID NO: 3 or a variant thereof that has 95% identity to SEQ ID NO: 3. In other words, the protein claimed may be larger than SEQ ID NO: 3 or its variant with 95% identity to SEQ ID NO: 3. It should be noted that "having" is open language, equivalent to "comprising".

The claim has two different generic embodiments, the first being a protein which comprises SEQ ID NO: 3 and the second being variants of SEQ ID NO: 3. There is a single species disclosed, that species being SEQ ID NO: 3.

A search of the prior art indicates that SEQ ID NO: 3 is novel and unobvious.

There is actual reduction to practice of the single disclosed species. The specification indicates that the genus of proteins that must be variants of SEQ ID NO: 3 does not have substantial variation since all of the variants must possess the specified catalytic activity and must have at least 95% identity to the reference sequence, SEQ ID NO: 3. The single species disclosed is representative of the genus because all members have at least 95% structural identity with the reference compound and because of the presence of an assay which applicant provided for identifying all of the at least 95% identical variants of SEQ ID NO: 3 which are capable of the specified catalytic activity. One of skill in the art would conclude that

applicant was in possession of the necessary common attributes possessed by the members of the genus.

Conclusion: The disclosure meets the requirements of 35 USC §112 first paragraph as providing adequate written description for the claimed invention.